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Original Article

Common variant within the FTO gene, rs9939609, obesity and type 2 diabetes in population of Karachi, Pakistan

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ABSTRACT

Aim: To determine the effect of genetic variants within the FTO gene (rs9939609) on obesity related traits and type 2 diabetes in South Asian population of Karachi, Pakistan.
Methods: A case-control study was conducted at Baqai Institute of Diabetology and Endocrinology (BIDE), Baqai Medical University situated in Karachi. A total of 296 patients with known type 2 diabetes and 198 controls aged greater than and equal to 45 years were recruited. The Anthropometric, clinical and biochemical data was collected on a structured questionnaire. Single nucleotide polymorphism (SNP) in FTO gene was identified by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR). Association between the single nucleotide polymorphism and categorical variables such as type 2 diabetes and obesity category was tested through logistic regression analysis.
Results: We observed a strong association of the minor allele A at rs9939609 with type 2 diabetes. Significant difference was observed in frequency of FTO genotype when diabetic subjects were compared with controls in co dominant, dominant and recessive models. This association remained significant even after adjusting for body mass index (BMI) and for waist circumference. The frequency of homozygous risk Alleles (AA) was found to be higher in obese & overweight ($\geq$23 kg/m^2) and females with central obesity in our study population. The association of FTO variant with BMI and central obesity does not reach to statistical significance.
Conclusion: In the study population of South Asian ancestry, variants of the FTO gene predispose to type 2 diabetes, but not entirely through their effect on BMI.

1. Introduction

Type 2 diabetes (T2DM) is a major health concern worldwide. Formerly, considered the disease of western countries only, now virtually exists in almost every nation of the world [1]. The prevalence of T2DM has reached an alarming rate during the last four decades. Asia is one of the epicenters of T2DM, accounting for 60% of the global population with diabetes [2]. It has been estimated that prevalence of impaired glucose tolerance and diabetes is high in Asian countries and the figure is expected to increase in the following years. Pakistan has an estimated 6.7 million people affected with diabetes, according to the International Diabetes Federation (IDF) and this number is predicted to increase to 12.8 million by the year 2035 [3].

High rates of obesity among children and women and association of obesity with metabolic risk including diabetes have been observed. The South Asian population particularly has shown to have one of the greatest risks [4]. Approximately 25% subjects aged 15 years or above is already overweight or obese in Pakistan. The changes in lifestyle are not limited to urban areas but rural population is also showing a similar trend worldwide, Pakistan being no exception. A community based survey reported a two-fold increase in the prevalence of diabetes and impaired fasting

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glucose as a result of significant increase in obesity [5]. Similar pattern of metabolic derangements leading to T2DM were observed in young adults aged 15–25 years [6].

Fat mass and obesity associated (FTO) gene was the first reported obesity and diabetes susceptibility locus [7]. Significant association of FTO gene with BMI and obesity risk has been evidenced in different European, East Asian and south Asian populations [8–10]. A GWAS of 7861 Koreans identified FTO gene as the most significantly associated obesity gene [11]. Likewise, a meta analyses in Asians reported significant relationship of FTO variants with increased risk of obesity [10].

Since the data highlighting the association of FTO gene with obesity related traits and T2DM in the South Asian population of Karachi, Pakistan is scarce; therefore we aimed to see the effect of genetic variants within the FTO gene (rs9939609) on obesity related traits and T2DM.

2. Materials and methods

This case-control study was conducted at Baqai Institute of Diabetology and Endocrinology (BIDE), Baqai Medical University situated in Karachi, Pakistan. The study duration was from March, 2011 to May, 2013. Karachi, the largest metropolitan city in Pakistan having representation of all major ethnic groups [12]. BIDE, a well-known diabetes care setup situated in the central zone of the city having access from all districts of Karachi is serving as a private tertiary care medical unit. Type 2 diabetic subjects across the city area with various socioeconomic group report at this center for their medical management.

Ethical approval for the study was obtained from an Institutional Review Board (IRB) of BIDE. Participation of the individuals was voluntary and they were included in the study after obtaining signed informed consent. A total of 296 patients with known T2DM and 198 control subjects were recruited and their data collected on a structured questionnaire. Diabetic patients meeting the inclusion criterion were recruited on a consecutive basis. Simple random sampling without replacement technique was used to collect samples. Control subjects were recruited from community screening camps set up at leading medical care units of Karachi. The control group in the study comprised of related or unrelated healthy individuals. Normal glucose tolerance was defined as fasting plasma glucose of $<$100 mg/dl. Diabetes was defined as fasting plasma glucose $>$126 mg/d) or taking anti-diabetes medication.

All patients with known T2DM, aged $\geq$ 45 years were considered eligible to participate in the study. Patients with T2DM with severe cardiac, renal and hepatic diseases, patients with type 1 diabetes, pregnant females and hospitalized individuals with T2DM were excluded from the study. Non diabetic people aged $\geq$ 45 years having no previous history of impaired fasting glucose or diabetes was included in control pool of the study [13].

Measurements of waist circumference (WC), Hip circumference (HC), Waist to hip ratio (WHR), BMI, blood pressure, fasting analytes including venous plasma glucose, serum cholesterol, triglycerides (TG), low-density lipoprotein (LDL) cholesterol, HDL cholesterol, HbA1c and serum creatinine were performed as per standard procedures [14–18].

As per guidelines for the Asian population, BMI was categorized into normal weight between 18 and 22.9 kg/m^2, overweight between 23 and 24.9 kg/m^{2}and obese $\geq$25 kg/m^2 [19]. Central obesity was defined as waist circumference $\geq$90 cm in men and $\geq$80 cm in women and WHR of 0.9 in males and 0.85 in females [20,21]. Hypertension was diagnosed if blood pressure was $\geq$130/85 mmHg or if the participants were taking anti-hypertensive medication or if they had self-reported history of hypertension [22,23].

DNA was extracted from whole blood by Phenol-Chloroform Method (an in-house DNA extraction method) [24]. Single nucleotide polymorphism (SNP) in FTO gene was identified by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) [25]. Primer 3 software was employed for designing outer and inner pairs of primer specifically designed for FTO SNP (rs 9939609) detection.

Data analysis was conducted on Statistical Package for Social Sciences (SPSS), version 13.0. All the continuous variables, i.e. age, duration of diabetes, weight, height, body mass index (BMI), systolic and diastolic blood pressure, fasting plasma glucose, HbA1c, presented as Mean $\pm$ SD.

T-test was utilized to find the difference in mean values and Chi Square Test of Independence used for categorical variables. $P < 0.05$ was considered as statistically significant. Hardy Weinberg equilibrium test (HWE) was applied to determine the variation in distribution of alleles and genotypes within the concerned population.

Association between the single nucleotide polymorphism and categorical variables such as T2DM and obesity category was tested using logistic regression.

3. Results

We compared the distribution of genotypes in 296 T2DM patients and 198 non-diabetic controls. Table 1 shows the comparison of anthropometric, clinical and biochemical characteristics of the study population. Patients with T2DM had significantly higher weight, waist circumference and waist to hip ratio in comparison to control participants ($P < 0.05$). Patients with T2DM had significantly higher systolic blood pressure, FPG and serum triglyceride while HDL level is found significantly lower in patients with T2DM as compared to controls ($P < 0.05$).

The frequency of overweight, obesity and central obesity (females) was significantly higher in patients with T2DM as compared to control subjects ($P < 0.05$) (Table 2).

The "A" allele at rs9939609 in the FTO gene had a frequency (MAF) of 0.48 and 0.74 in controls and diabetics respectively. The genotypes were in Hardy–Weinberg equilibrium. The frequency of

Table 1
Comparison of baseline characteristics of study population.

Characteristics $n = 494$	Patients with T2DM $n = 296$	Control subjects $n = 198$	*P*-value
Age (years)	49.58 ± 10.38	51.46 ± 7.68	0.031
Male (%)	179 (60.5%)	83 (42.1%)	<0.0001
Female (%)	117 (39.5%)	114 (57.9%)	
Weight (kg)	75.03 ± 16.67	70.60 ± 13.61	0.002
Body mass index (kg/m^2)	28.89 ± 5.27	28.08 ± 7.86	0.177
Waist circumference male (cm)	96.08 ± 10.61	93.12 ± 12.59	0.054
Waist circumference female (cm)	98.57 ± 12.79	88.22 ± 12.40	<0.0001
Waist to hip ratio (Male)	0.96 ± 0.06	0.90 ± 0.08	<0.0001
Waist to hip ratio (Female)	0.89 ± 0.08	0.85 ± 0.08	0.001
Systolic blood pressure (mmHg)	127.88 ± 17.63	115.28 ± 14.14	0.000
Diastolic blood pressure (mmHg)	79.47 ± 9.59	79.79 ± 11.29	0.735
HbA1c (%)	9.34 ± 1.96	–	–
Fasting plasma glucose (mg/dl)	183.36 ± 77.72	89.24 ± 9.93	0.000
Serum cholesterol (mg/dl)	178.67 ± 45.78	177.24 ± 37.61	0.739
Triglycerides (mg/dl)	182.33 ± 162.71	125.04 ± 76.74	0.000
High density lipoprotein (mg/dl)	38.50 ± 10.47	42.55 ± 10.21	0.000
Low density lipoprotein (mg/dl)	101.33 ± 34.56	111.71 ± 27.97	0.001

Data presented as Mean ± SD and *n* (%).
$P < 0.05$ considered as statistically significant.